

# PATENT COOPERATION TREATY

## PCT

### NOTIFICATION OF THE RECORDING OF A CHANGE

(PCT Rule 92bis.1 and  
Administrative Instructions, Section 422)

From the INTERNATIONAL BUREAU

To:

WILSON, Kathryn, S.  
Level 12  
KPMG Centre, 85 Alexandra Street  
Private Bag 3140  
Hamilton  
NOUVELLE-ZÉLANDE

<b>Date of mailing (day/month/year)</b> 10 July 2001 (10.07.01)	
<b>Applicant's or agent's file reference</b> 41254PCX110	<b>IMPORTANT NOTIFICATION</b>
<b>International application No.</b> PCT/NZ00/00159	<b>International filing date (day/month/year)</b> 17 August 2000 (17.08.00)

1. The following indications appeared on record concerning: <input checked="" type="checkbox"/> the applicant <input type="checkbox"/> the inventor <input type="checkbox"/> the agent <input type="checkbox"/> the common representative		
<b>Name and Address</b> NEW ZEALAND PASTORAL AGRICULTURE RESEARCH INSTITUTE LIMITED East Street Ruakura Campus Hamilton New Zealand	<b>State of Nationality</b> NZ	<b>State of Residence</b> NZ
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2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning: <input checked="" type="checkbox"/> the person <input type="checkbox"/> the name <input checked="" type="checkbox"/> the address <input type="checkbox"/> the nationality <input type="checkbox"/> the residence		
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3. Further observations, if necessary:		
4. A copy of this notification has been sent to: <div style="display: flex; justify-content: space-between;"> <div> <input checked="" type="checkbox"/> the receiving Office  <input type="checkbox"/> the International Searching Authority  <input type="checkbox"/> the International Preliminary Examining Authority                         </div> <div> <input type="checkbox"/> the designated Offices concerned  <input checked="" type="checkbox"/> the elected Offices concerned  <input type="checkbox"/> other:                         </div> </div>		

<b>The International Bureau of WIPO</b> 34, chemin des Colombettes 1211 Geneva 20, Switzerland	<b>Authorized officer</b> <div style="text-align: center;">A. Karkachi</div>
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<b>Name and Address</b> AGRESEARCH LIMITED 5th Floor Tower Block Ruakura Research Centre East Street Hamilton, 2001 New Zealand	<b>State of Nationality</b> NZ	<b>State of Residence</b> NZ
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<b>3. Further observations, if necessary:</b>		
<b>4. A copy of this notification has been sent to:</b> <div style="display: flex; justify-content: space-between;"> <div> <input checked="" type="checkbox"/> the receiving Office  <input type="checkbox"/> the International Searching Authority  <input type="checkbox"/> the International Preliminary Examining Authority         </div> <div> <input type="checkbox"/> the designated Offices concerned  <input checked="" type="checkbox"/> the elected Offices concerned  <input type="checkbox"/> other:         </div> </div>		

<b>The International Bureau of WIPO</b> 34, chemin des Colombettes 1211 Geneva 20, Switzerland  Facsimile No.: (41-22) 740.14.35	<b>Authorized officer</b>  A. Karkachi  Telephone No.: (41-22) 338.83.38
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**PATENT COOPERATION TREATY**

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**NOTIFICATION OF THE RECORDING  
OF A CHANGE**

(PCT Rule 92bis.1 and  
Administrative Instructions, Section 422)

From the INTERNATIONAL BUREAU

To:

WILSON, Kathryn, S.  
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<b>Date of mailing</b> (day/month/year) 27 April 2001 (27.04.01)	<b>IMPORTANT NOTIFICATION</b>
<b>Applicant's or agent's file reference</b> 41254PCX110	
<b>International application No.</b> PCT/NZ00/00159	<b>International filing date</b> (day/month/year) 17 August 2000 (17.08.00)

1. The following indications appeared on record concerning:

☐ the applicant      ☐ the inventor      ☒ the agent      ☐ the common representative

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3. Further observations, if necessary:

**The new agent's address on the Demand has been considered as a change under Rule 92bis.  
In case of disagreement, the International Bureau should be notified immediately.**

4. A copy of this notification has been sent to:

☒ the receiving Office      ☐ the designated Offices concerned  
☐ the International Searching Authority      ☒ the elected Offices concerned  
☒ the International Preliminary Examining Authority      ☐ other:

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## PATENT COOPERATION TREATY

PCT

## INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

REC'D 03 MAY 2001

WIPO

PCT

14

Applicant's or agent's file reference 41254/20jm	<b>FOR FURTHER ACTION</b>	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416).
International Application No. <b>PCT/NZ00/00159</b>	International Filing Date (day/month/year) 17 August 2000	Priority Date (day/month/year) 17 August 1999
International Patent Classification (IPC) or national classification and IPC  Int. Cl. <sup>7</sup> C12Q 1/34, 1/54		
Applicant  NEW ZEALAND PASTORAL AGRICULTURE RESEARCH INSTITUTE LIMITED et al		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 3 sheets, including this cover sheet.
- ☐ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consist of a total of sheet(s).

3. This report contains indications relating to the following items:

- |      |                                     |   |
|------|-------------------------------------|---|
| I    | <input checked="" type="checkbox"/> | Basis of the report   |
| II   | <input type="checkbox"/>            | Priority  |
| III  | <input type="checkbox"/>            | Non-establishment of opinion with regard to novelty, inventive step and industrial applicability  |
| IV   | <input type="checkbox"/>            | Lack of unity of invention  |
| V    | <input checked="" type="checkbox"/> | Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement |
| VI   | <input type="checkbox"/>            | Certain documents cited   |
| VII  | <input type="checkbox"/>            | Certain defects in the international application  |
| VIII | <input type="checkbox"/>            | Certain observations on the international application   |

Date of submission of the demand 9 March 2001	Date of completion of the report 18 April 2001
Name and mailing address of the IPEA/AU AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaaustralia.gov.au Facsimile No. (02) 6285 3929	Authorized Officer  <b>PHILIPPA WYRDEMAN</b> Telephone No. (02) 6283 2554

**I. Basis of the report**

1. With regard to the **elements** of the international application:\*
- ☒ the international application as originally filed.
- ☐ the description,        pages , as originally filed,  
   pages , filed with the demand,  
   pages , received on    with the letter of
- ☐ the claims,                pages , as originally filed,  
   pages , as amended (together with any statement) under Article 19,  
   pages , filed with the demand,  
   pages , received on    with the letter of
- ☐ the drawings,            pages , as originally filed,  
   pages , filed with the demand,  
   pages , received on    with the letter of
- ☐ the sequence listing part of the description:  
   pages , as originally filed  
   pages , filed with the demand  
   pages , received on    with the letter of
2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.  
These elements were available or furnished to this Authority in the following language    which is:
- ☐ the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of the translation furnished for the purposes of international preliminary examination (under Rules 55.2 and/or 55.3).
3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, was on the basis of the sequence listing:
- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished
4. ☐ The amendments have resulted in the cancellation of:
- ☐ the description,        pages
- ☐ the claims,                Nos.
- ☐ the drawings,            sheets/fig.
5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).\*\*

\* Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).

\*\* Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report

**V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

## 1. Statement

Novelty (N)	Claims 1-23	YES
	Claims None	NO
Inventive step (IS)	Claims 1-23	YES
	Claims None	NO
Industrial applicability (IA)	Claims 1-23	YES
	Claims None	NO

## 2. Citations and explanations (Rule 70.7)

Novelty (N)

All the documents cited in the ISR were category A only. Therefore the claimed invention is not disclosed in any of these patent documents and hence all the claims are novel.

Inventive Step (IS)

The claimed invention is not obvious in the light of any of the cited documents nor disclosed in any obvious combination, nor would the claimed invention be obvious to a person skilled in the art in the light of common general knowledge by itself or in combination with any of these documents.

Industrial Applicability (IA)

The claimed material is considered Industrially Applicable.

(19) World Intellectual Property Organization  
International Bureau



(43) International Publication Date  
22 February 2001 (22.02.2001)

PCT

(10) International Publication Number  
**WO 01/12844 A1**

(51) International Patent Classification<sup>7</sup>: C12Q 1/34, 1/54

(21) International Application Number: PCT/NZ00/00159

(22) International Filing Date: 17 August 2000 (17.08.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:  
337276 17 August 1999 (17.08.1999) NZ

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(81) Designated States (national): AE, AG, AL, AM, AT, AU,  
AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ,  
DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR,  
HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR,  
LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ,  
NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM,  
TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States (regional): ARIPO patent (GH, GM,  
KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian  
patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European  
patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE,  
IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG,  
CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:

— With international search report.

For two-letter codes and other abbreviations, refer to the "Guid-  
ance Notes on Codes and Abbreviations" appearing at the begin-  
ning of each regular issue of the PCT Gazette.

(54) Title: RAPID METHOD FOR MEASURING COMPLEX CARBOHYDRATES IN MAMMALIAN TISSUE

(57) Abstract: The invention discloses a rapid method of measuring complex carbohydrates in mammalian tissue, said method comprising the steps of: extracting a sample of tissue to be tested; forming a homogenous slurry of the sample with an aqueous solution; adding sufficient hydrolysing enzyme for ensuring complete hydrolysis of glycogen in the slurry; and measuring the concentration of glucose in the slurry. The method can be conducted post-mortem to assay the concentration at the time of slaughter. The invention further discloses the measurement of lactate concentrations. The method of the invention can be conducted in thirty minutes or less.

WO 01/12844 A1

**TITLE: RAPID METHOD FOR MEASURING COMPLEX CARBOHYDRATES  
IN MAMMALIAN TISSUE**

**TECHNICAL FIELD**

5 The present invention relates to a rapid method for measuring complex carbohydrates, particularly glycogen, in mammalian tissue. More particularly the present invention relates to the rapid measurement of glycogen in non-living mammalian tissue.

**BACKGROUND ART**

10 There are presently a number of methods of measuring complex carbohydrates, and particularly glycogen, in mammalian tissue. The discussion of these follows. The relevance of the measurement of glycogen includes, for example, the ability to use the results of glycogen measurement as a determination of the ultimate pH of meat. This in turn is a direct measure of many of the qualities of meat.

15 There are a number of known methods of measuring ultimate pH in meat: including use of liquid nitrogen in a freeze/thaw process and the use of a pH electrode for pH determination. There are a number of variations of this method, also. However the maintenance and use of liquid nitrogen in the quantities needed for the measurement on a continuous series of carcasses reveals hazards for the work environment. Also there is some doubt as to the  
20 accuracy and consistency of such measurement methods.



## Methods of Measurement of Glycogen or Metabolites in Meat Samples

The iodine method: The principle of this method is that glycogen will react with a mixture of iodide, iodine and calcium chloride, forming an amber pigment in acid solution that has a linear absorption at least over a small, specified range. The glycogen is extracted from the meat with perchloric acid that is then filtered and centrifuged to recover a solution of glycogen which is reacted with the iodine. The extraction can also be by liquid nitrogen, potassium hydroxide, ethanol and ammonium chloride.

However, methods of extraction and then assay are time-consuming and employ aggressive chemical reagents.

Hydrolysis of glycogen with enzymes: The principle of this method is that glycogen hydrolyses to glucose, after which standard methods of measurement of free glucose may be used. The amyloglucosidase method of Dreiling et al (Meat Science, Vol 20, p. 167) is one such method, although other enzymes may be used. A muscle sample is homogenised with perchloric acid, and centrifuged, The supernatant, containing dissolved glycogen, is neutralised. Amyloglucosidase is added, converting the glycogen to glucose, for measurement. The first part of the method takes approximately 30 minutes at 37°C.

However there are some instances with the processing of meat in which a test of some 30 minutes or more is too long a time to wait for the test results, and some reagents are aggressive.

It is an object of the present invention to provide a rapid method for glycogen measurement in mammalian tissues, and in particular non-living tissue. It is a further object of the present

invention that in addition to the provision of a rapid accurate measurement of glycogen in tissue, the method also utilise mild or non-aggressive reagents.

Whilst the prior art and uses of glycogen measurement have been described with reference to a determination of the ultimate pH in meat, it will be appreciated that this method is not limited thereto. For example, low glycogen levels in the liver of bobby calves are an indicator of inadequate feeding before slaughter. Thus such rapid method of assessment of these levels could assist in ensuring good animal welfare.

It is an object of the present invention to address the foregoing problems or at least to provide the public with a useful choice.

Further aspects and advantages of the present invention will become apparent from the ensuing description which is given by way of example only.

#### DISCLOSURE OF INVENTION

For the purposes of this specification, the term "rapid" is used to refer to times of less than 30 minutes and, more preferably, significantly less than 30 minutes

According to one aspect of the present invention there is provided a rapid method of measuring complex carbohydrates in mammalian tissue, said method comprising the steps of:

- (a) extracting a sample of tissue to be tested;
- (b) forming a homogenous slurry of the sample with an aqueous solution;
- (c) adding sufficient hydrolysing enzyme for ensuring complete hydrolysis of

glycogen in the slurry; and

(d) measuring the concentration of glucose in the slurry.

Advantageously, said complex carbohydrate is glycogen. Advantageously, said aqueous solvent is water. Optionally said solvent may include at least one agent intended to standardise ionic conditions and/or facilitate the steps of the above method.

Preferably the formation of the homogenous slurry is effected with a high or a low speed homogeniser, or with an ultrasonic apparatus.

According to another aspect of the present invention there is provided a rapid method of measuring complex carbohydrates in mammalian tissue, substantially as described above, wherein steps (b) and (c) are performed simultaneously.

According to another aspect of the present invention there is provided a rapid method of measuring complex carbohydrates in mammalian tissue, substantially as described above, wherein steps (c) and (d) are performed simultaneously.

Advantageously, the hydrolysing enzyme may be any enzyme, or combination of enzymes, capable of hydrolysis of glycogen to glucose.

According to a still further aspect of the present invention there is provided a rapid method of measuring complex carbohydrates in mammalian tissue, substantially as described above, in which said hydrolysing enzyme is selected from the group: amyloglucosidase;  $\alpha$ -amylase;  $\alpha$ -glucosidase, and a combination thereof.

According to another aspect of the present invention there is provided a rapid method of measuring complex carbohydrates in mammalian tissue, substantially as described above,

wherein the amyloglucosidase added in step (c) is in a form selected from: a powder; a liquid suspension; and a solution.

According to another aspect of the present invention there is provided a rapid method of measuring complex carbohydrates in mammalian tissue, substantially as described above, in either embodiment, wherein said method further includes a step (e): measuring the concentration of lactate in the sample.

According to another aspect of the present invention there is provided a rapid method of measuring complex carbohydrates in mammalian tissue, substantially as described above, wherein steps (d) and (e) are performed simultaneously.

According to another aspect of the present invention there is provided a rapid method of measuring complex carbohydrates in mammalian tissue, substantially as described above, wherein steps (c), (d) and (e) are performed simultaneously.

The measurement of both metabolites gives a good post-mortem estimate of the concentration of glycogen present in tissue at the time of death, no matter when the measurement is made.

Advantageously, the method is conducted at approximately room temperature, although it may be conducted at a temperature in the range 0°C to 100°C.

#### **Measurement of Glucose**

There are a range of methods for measuring glucose. For the present invention the most useful are those adapted from known technologies to measure glucose in blood. These are

usually based on the generation of hydrogen peroxide in stoichiometric proportion to glucose, as catalysed by glucose oxidase.

Therefore, according to a still further aspect of the present invention there is provided a rapid method of measuring complex carbohydrates in mammalian tissue, substantially as described  
5 above, and in which glycogen levels are measured and in which said measurement of the concentration of glucose is achieved by the construction of sensors incorporating said hydrolysing enzyme and glucose oxidase.

#### **Measurement of Lactate**

10 There are a range of methods for measuring lactate, including the standard NADH-linked method and those based on the generation of hydrogen peroxide in stoichiometric proportion to lactate, as catalysed by lactate oxidase. This latter compound can be incorporated into any sensor which may already incorporate glucose oxidase.

#### **15 BRIEF DESCRIPTION OF THE DRAWINGS**

Further aspects of the present invention will become apparent from the following example, which is given by way of example only, and with reference to the accompanying drawings in which:

Figure 1 is a graph of glucose concentration and glucometer reading of a glucose  
20 sample in acetate buffer;

Figure 2 is a graph of the glucometer reading for glucose and concentration of glucose

added to meat slurry samples;

Figure 3 is a graph of the kinetics of glucose formation from glycogen in acetate buffer in the present of amyloglucosidase;

Figure 4 is a graph of glucometer readings and the concentration of glucose added to  
5 a meat/acetate buffer slurry;

Figure 5 is a graph of glucose value and glycogen added to a post rigor meat slurry, at 5 minutes incubation at 55 °C;

Figure 6 is a graph of muscle glycogen concentration in post slaughter samples, using the method of measurement of the present invention;

10 Figure 7 is a graph showing the kinetics of glycogen loss, pH fall and lactate increase in a bovine muscle sampled after slaughter; and

Figure 8 is a graph of the repeatability of glycogen determination by the method of the present invention.

## 15 **BEST MODES FOR CARRYING OUT THE INVENTION**

### **Chemicals, Equipment and Meat.**

Amyloglucosidase from the fungus *Aspergillus niger*, in powder form, was added to meat slurries as a liquid suspension or solution. In suspension, 25 mg amyloglucosidase was dissolved in 5 ml of 3.2M ammonium sulphate and adjusted to pH 6.0 with ammonia. This  
20 particular solvent is known to be one in which the enzyme is stable. As an alternative, a clear solution can be obtained by the use of 25 mg of powder, dissolved in 5 ml of 0.2M sodium

acetate at a pH of 4.5.  $\alpha$ -D-Glucose of a standard analytical grade was used.

Glucose measurement was made with an Esprit glucometer (Bayer). Test sensors used in the Esprit were from Bayer New Zealand Limited. These were used for one reading only then discarded.

- 5 The meat samples tested were obtained from the *longissimus lumborum* muscle of a beef carcass, obtained from a butcher. Pre-rigor muscle, variously *gluteus medius*, *semitendinosus* and *longissimus lumborum*, was obtained from an abattoir. These muscles were dissected from unstimulated carcasses approximately 25 minutes after slaughter and tested very shortly thereafter. The muscles were held at room temperature while measurements were made.

10

### Glycogen Test Procedure

The test medium for all experiments was 0.2M sodium acetate buffer at a pH of 4.4 and at a temperature of 55°C. In the experiments the muscle or meat (in samples of accurately known weight, but approximately 1 g) was homogenised in 5 ml of buffer, with a high speed  
15 Polytron shearing head. This was usually set at 25,000 rpm. Alternatively a lower speed homogeniser may be used, if so desired. The homogeniser may be a stainless steel paddle-like blade rotating at 2000 rpm in a steel cup within an interior shaped like a standard domestic Waring blender.

- After homogenisation the enzyme solution was added. The volume of this solution was  
20 usually 200  $\mu$ l, containing 1 mg of amyloglucosidase. The mixture was briefly shaken, then held at 55°C.

Small aliquots, of approximately 20  $\mu$ l, were withdrawn at intervals with disposable pipettes,

and spotted onto plastic film. The glucometer sensor sampled these drops and returned a meter reading for glucose concentration in 30 seconds.

As a control, tests were also carried out with a range of glucose and glycogen concentrations in an acetate buffer to which no meat or meat samples had been added.

5

### Lactate Measurement

In one range of experiments lactate concentration was also measured. At various times after slaughter, a crude aliquot of the slurry, containing homogenised muscle and amyloglucosidase was centrifuged in a micro-centrifuge (at 10, 000 rpm for 30 seconds).

10 The clear supernatant was recovered and analysed for lactate concentration by the NADH-linked method.

### Results

The results from the glucometer were in mg of glucose/dl. These results are tabulated in Fig. 1 of the attached drawings. It is noted that the relation was linear but that the readings were approximately double the glucose concentration in fact present and did not pass through the origin.

The exact reason for the approximate doubling of the readings is not known. However it is understood that this might relate to the characteristics of glucose in blood.  $\alpha$ -D-glucose as a laboratory chemical dissolved in acetate at a pH of 4.5 may require a different calibration from that in blood. However, the failure of the straight line to pass through the origin



suggests that the acetate medium affected the sensor performance.

### Example 1

Various samples of meat (the samples being as described above) between 0.90 and 1.16 g  
5 were homogenised in the high speed homogeniser in 5 ml of acetate buffer containing up to  
16 mg of added glucose. This addition rate translates to approximately 267 mg/dl, assuming  
the density of meat is about 1 g/ml.

As can be seen from Fig. 2 of the attached drawings the relationship, the relationship between  
added glucose and the average meter reading is linear. The positive value of detected glucose  
10 with zero added glucose may be explained by the small quantities of glucose left over from  
glycolysis in the meat samples.

### Example 2

The method of Example 1 was repeated over a range of samples and concentrations in which  
15 various quantities of glycogen (between 0 - 14 mg) were added to 5 ml of acetate buffer and  
the reactions were started by amyloglucosidase addition.

The results are as set out in Fig. 3 of the attached drawings, in which can be seen the glucose  
values peaked and declined slightly. The reason for the decline is not understood but it is  
possible that this may result from a contaminating activity in the enzyme preparation or from  
20 isomerisation reactions of glucose liberated from glycogen.

The data tabulated in Fig. 3 suggest that at even the highest concentrations of glycogen,

around 40 activity units of amyloglucosidase, are sufficient to fully hydrolyse the glycogen within approximately 5 minutes.

### Example 3

- 5 The experiment the results of which are tabulated in Fig. 3 was repeated in the presence of rigor meat. Meat samples ranged from between 0.97 - 1.07 g in six tests. The results are as set out in Fig. 4 of the attached drawings.

Individual data points were abstracted to plot glycogen added against meter readings for 5 minutes after the amyloglucosidase addition. The results of this are shown in Fig. 5. A

- 10 quadratic equation was fitted, but the shape was close to a straight line.

### Example 4

Samples of *gluteus medius* and a *semitendinosus* muscle were removed from pre-rigor meat, cut to samples in the size between 0.95 - 1.05 g, and homogenised in 5 ml of acetate buffer.

- 15 This slurry was then treated with amyloglucosidase. The tests began 1.3 hours after slaughter, first with the *semitendinosus* muscle and at that time the muscle contained only 3.8 mg glycogen /g. By 2 hours the glycogen level declined to 1 mg/g.

In contrast, *gluteus medius* muscle sample contained 13.1 mg/g at 2.3 hours post slaughter, and the value declined steadily with time. The results are as shown in Fig. 6.

- 20 This experiment was repeated using *longissimus lumborum* muscle from another animal. This muscle is frequently used as an indicator of high pH condition. Lactate concentrations

were also measured.

The above tests as described were carried out and the results are as shown in Fig. 7 of the attached drawings. In this method the measurement of lactate was as per the standard NADH-linked method rather than the use of a sensor based on lactate oxidase.

5

### Example 5

The robustness of the preferred embodiment of the test of the present invention was further determined by the following: meter readings were recorded in which triplicate aliquots of glycogen were added to three replicate rigor meat samples weighing  $1.0 \pm 0.05$  g. The  
10 slurry was treated in the same manner as described above for Example 2. The results of this are set out in Fig. 8 of the attached drawings.

Whilst the Examples given above to show the best method of performing the invention are all with reference to meat samples (beef) it would be appreciated by those skilled in the arts that other tissue samples may be equally treated in like manner to produce glycogen  
15 measurement as a result.

Aspects of the present invention have been described by way of example only and it should be appreciated that modifications and additions may be made thereto without departing from the scope thereof.

Throughout this specification and the claims which follow, unless the context requires  
20 otherwise, the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integers or steps.

**CLAIMS**

1. A rapid method of measuring complex carbohydrates in mammalian tissue, said method comprising the steps of:

(a) extracting a sample of tissue to be tested;

5 (b) forming a homogenous slurry of the sample with an aqueous solution;

(c) adding sufficient hydrolysing enzyme for ensuring complete hydrolysis of glycogen in the slurry; and

(d) measuring the concentration of glucose in the slurry.

10 2. A rapid method of measuring complex carbohydrates as claimed in claim 1 wherein said method is performed in less than 30 minutes.

3. A rapid method of measuring complex carbohydrates as claimed in either claim 1 or claim 2 wherein said complex carbohydrate is selected from glycogen, lactate and a  
15 combination of these.

4. A rapid method of measuring complex carbohydrates as claimed in any one of the preceding claims wherein said aqueous solvent is water.

20 5. A rapid method of measuring complex carbohydrates as claimed in any one of the

preceding claims wherein said aqueous solvent includes at least one agent to standardise ionic conditions obtaining for the method.

6. A rapid method of measuring complex carbohydrates as claimed in any one of the preceding claims wherein the formation of the homogenous slurry is effected with apparatus selected from: a high speed homogeniser; a low speed homogeniser and an ultrasonic apparatus.

7. A rapid method of measuring complex carbohydrates as claimed in any one of the preceding claims wherein said hydrolysing enzyme is selected from the group: amyloglucosidase;  $\alpha$ -amylase;  $\alpha$ -glucosidase, and a combination thereof.

8. A rapid method of measuring complex carbohydrates as claimed in claim 7 wherein said hydrolysing enzyme is amyloglucosidase which is in a form selected from: a powder; a liquid suspension; and a solution.

9. A rapid method of measuring complex carbohydrates as claimed in any one of the preceding claims wherein said method further includes a step (e): measuring the concentration of lactate in the sample.

10. A rapid method of measuring complex carbohydrates as claimed in any one of the

preceding claims wherein steps (b) and (c) are performed simultaneously.

11. A rapid method of measuring complex carbohydrates as claimed in any one of claims 1 to 9 wherein steps (c) and (d) are performed simultaneously.

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12. A rapid method of measuring complex carbohydrates as claimed in claim 9 wherein steps (d) and (e) are performed simultaneously.

13. A rapid method of measuring complex carbohydrates as claimed in claim 9 wherein  
10 steps (c) to (e) are performed simultaneously.

14. A rapid method of measuring complex carbohydrates as claimed in claim 9 wherein said measurement of lactate concentration is by the NADH-linked method.

15 15. A rapid method of measuring complex carbohydrates as claimed in claim 9 wherein said measurement of lactate concentration is based on the generation of hydrogen peroxide in stoichiometric proportion to lactate, as catalysed by lactate oxidase.

16. A rapid method of measuring complex carbohydrates as claimed in any one of the  
20 preceding claims wherein said glycogen measurement is effected by use of sensors which

incorporate said hydrolysing enzyme and glucose oxidase.

17. A rapid method of measuring complex carbohydrates as claimed in claim 16 wherein said sensor further includes lactate oxidase.

5

18 A rapid method of measuring complex carbohydrates as claimed in any one of the preceding claims wherein said method is carried out at room temperature.

19. A rapid method of measuring complex carbohydrates as claimed in any one of the  
10 preceding claims wherein said method is performed post-mortem, providing a measurement of concentrations of complex carbohydrates at the time of death.

20. A rapid method of measuring complex carbohydrates as claimed in claim 19 wherein said method is performed wherein said measurement is up to half an hour after slaughter.

15

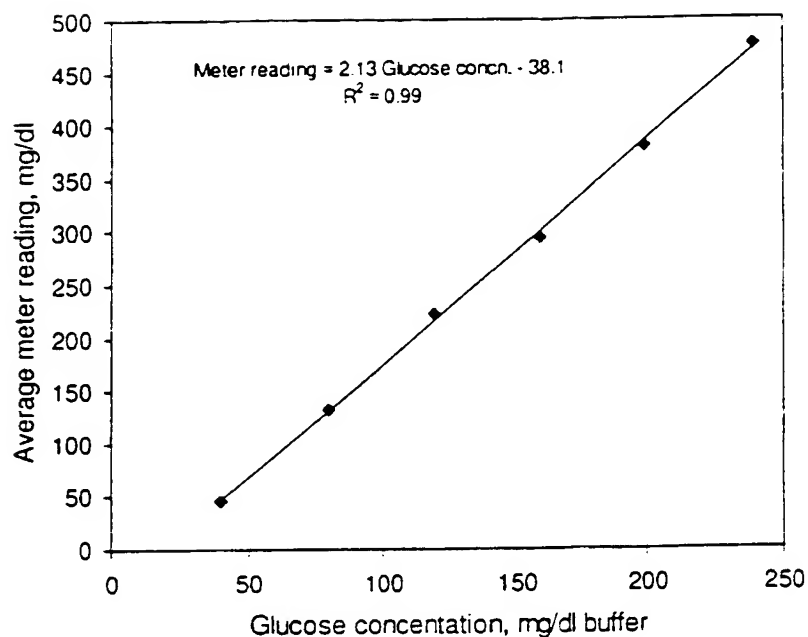
21. Measurement of ultimate pH by use of the method of measuring complex carbohydrates as claimed in any one of the preceding claims wherein said tissue is meat.

22. Measurement of ultimate pH by use of the method of measuring complex  
20 carbohydrates as claimed in any one of the preceding claims wherein said tissue is muscle.

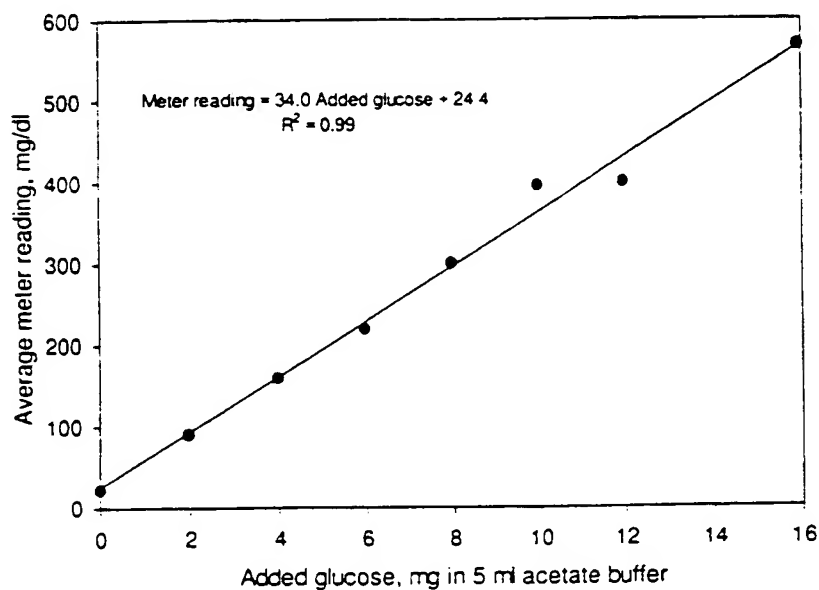
23. Measurement of ultimate pH by use of the method of measuring complex carbohydrates as claimed in claim 22 wherein said muscle is selected from: the *longissimus lumborum*; *gluteus medius*; *semitendinosus* and *longissimus lumborum* muscles.



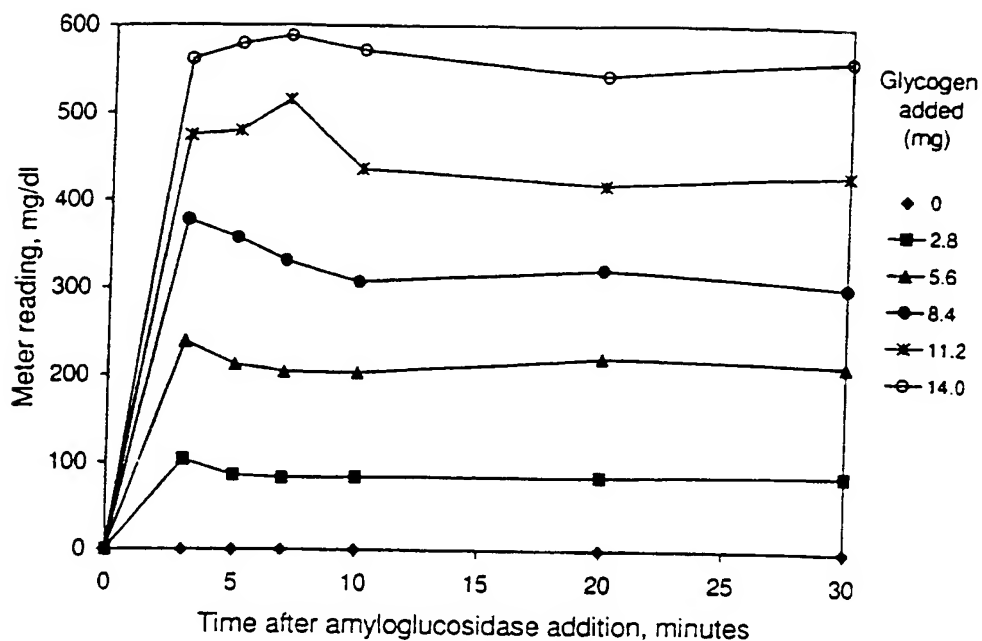
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**Figure 1**

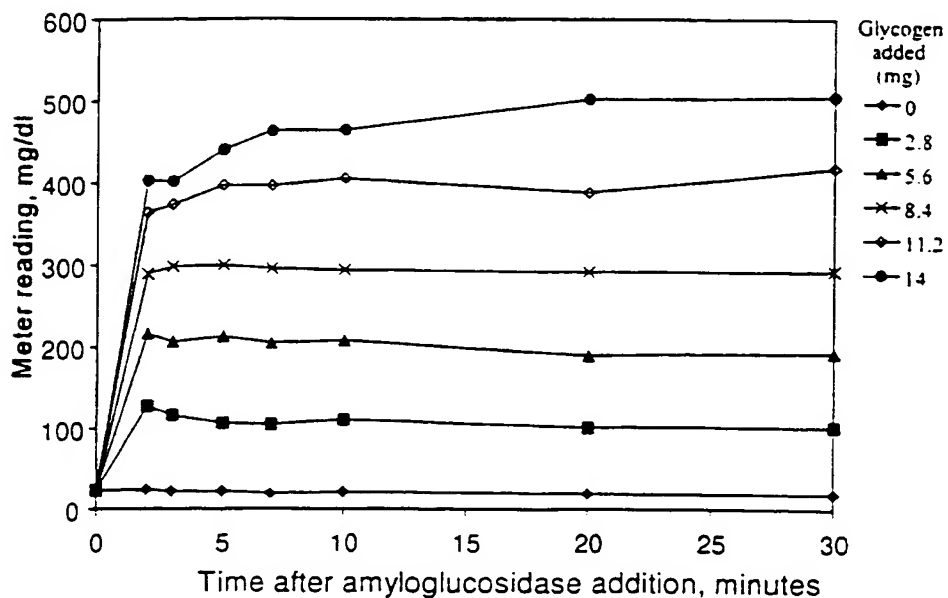
Relationship between the Esprit meter value for glucose and the concentration of  $\alpha$ -D-glucose in acetate buffer at pH 4.5. Points are means of duplicates.

**Figure 2**

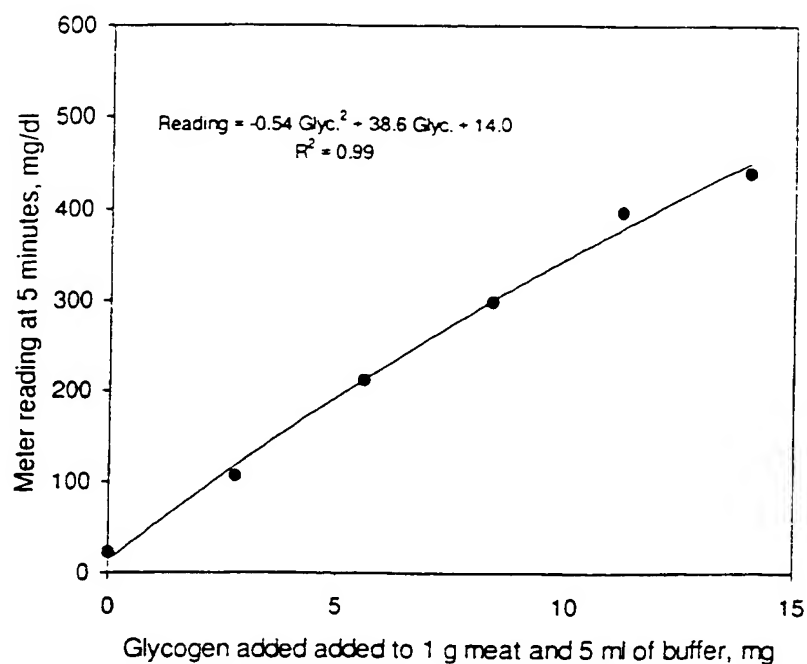
Relationship between the Esprit meter value for glucose and the concentration of  $\alpha$ -D-glucose in a meat/acetate buffer slurry at pH 4.5. Points are means of duplicates.

**Figure 3**

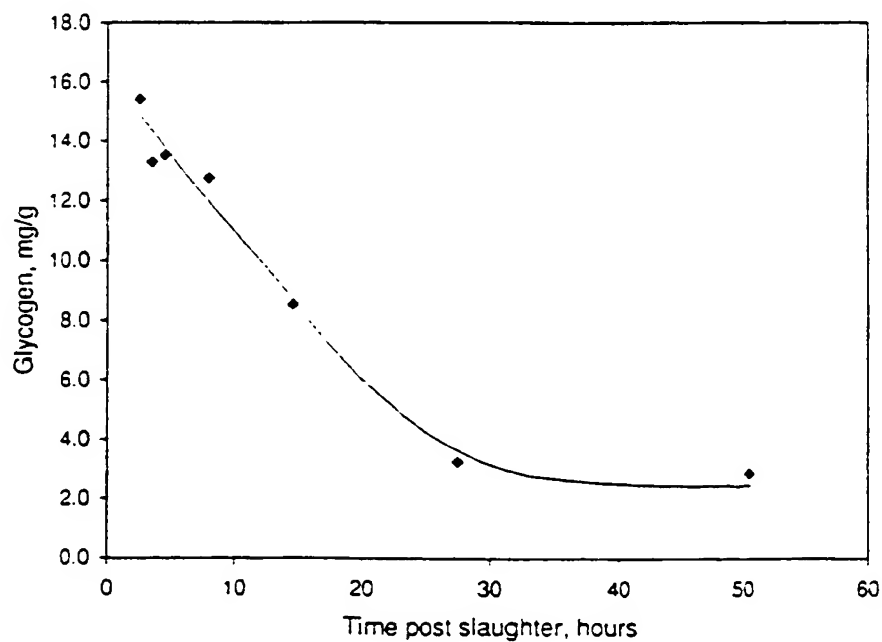
Kinetics of glucose formation from glycogen in acetate buffer in the presence of amyloglucosidase.

**Figure 4**

Kinetics of glucose formation from glycogen by amyloglucosidase in a meat slurry.

**Figure 5**

Relationship between the Esprit meter value for glucose and the quantity of glycogen in a post rigor meat slurry that also containing amyloglucosidase. Values were those recorded after 5 minutes incubation at 55°C. A quadratic equation was fitted to the data.

**Figure 6**

Kinetics of glycogen loss in *gluteus medius* as determined by the amyloglucosidase method.

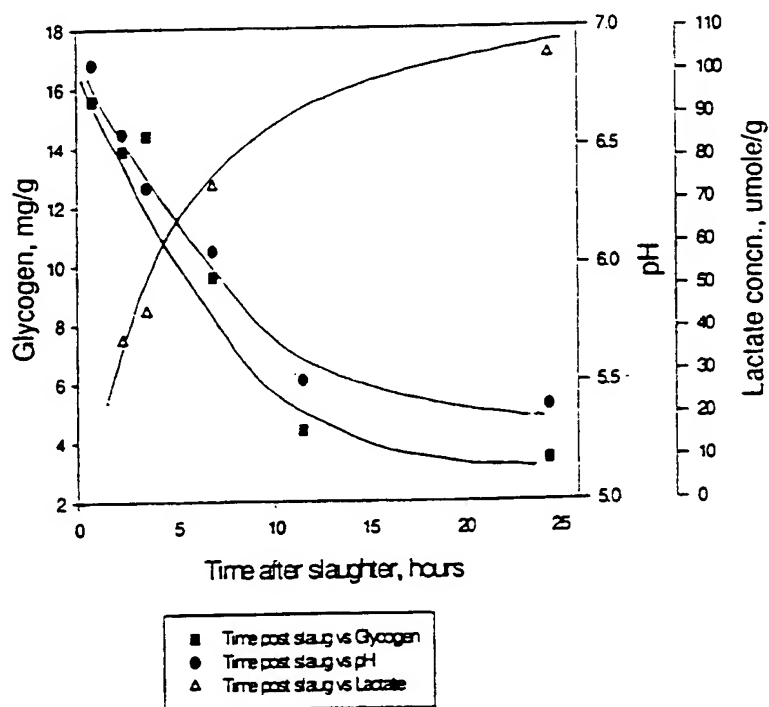
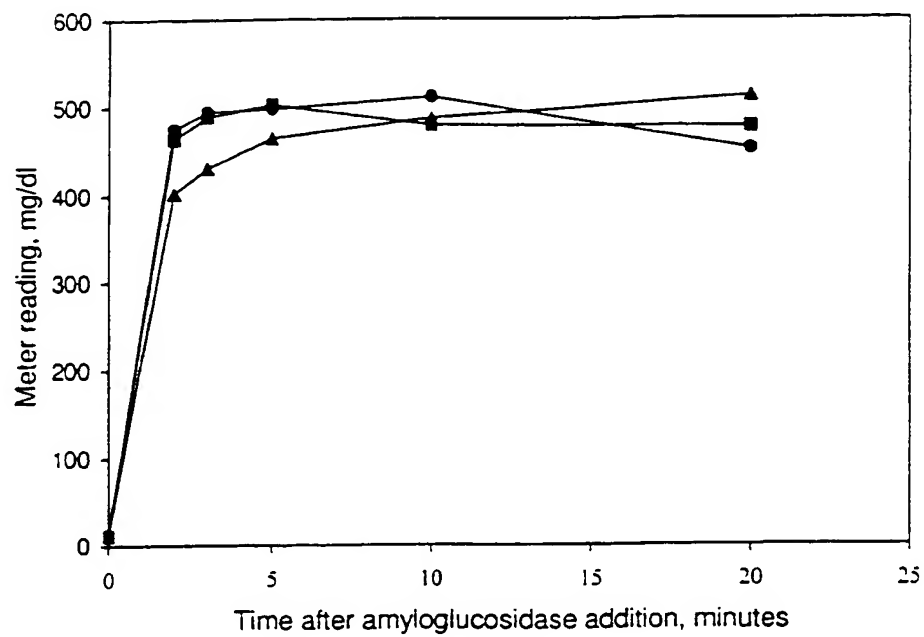


Figure 7

Kinetics of glycogen loss, pH fall and lactate increase in *longissimus lumborum* from an unstimulated carcass.



**Figure 8**

Repeatability of glycogen determination as glucose in the presence of rigor meat.

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/NZ00/00159

## A. CLASSIFICATION OF SUBJECT MATTER

Int. Cl. <sup>7</sup>: C12Q 1/34, 1/54

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)  
SEE ELECTRONIC DATABASE BOX BELOW

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched  
SEE ELECTRONIC DATABASE BOX BELOW

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Chem Abs, Medline, WPIDS:

Keywords: tissue, meat, muscle, hydrolysis, hydrolyzing, enzyme, glucose, glycogen.

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	CALDER, P. C. and GEDDES, R. (1990) "Post Mortem Glycogenolysis is a Combination of Phosphorolysis and Hydrolysis" <i>Int. J. Biochem.</i> Vol. 22, No. 8, pp. 847-856. See abstract part 2 in particular.	

☐ Further documents are listed in the continuation of Box C ☐ See patent family annex

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Date of the actual completion of the international search 2 November 2000	Date of mailing of the international search report 14 NOV 2000
Name and mailing address of the ISA/AU AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaaustralia.gov.au Facsimile No. (02) 6285 3929	Authorized officer  PHILIPPA WYRDEMAN Telephone No : (02) 6283 2554

(19) World Intellectual Property Organization  
International Bureau



(43) International Publication Date  
22 February 2001 (22.02.2001)

PCT

(10) International Publication Number  
**WO 01/12844 A1**

- (51) International Patent Classification<sup>7</sup>: C12Q 1/34, 1/54
- (21) International Application Number: PCT/NZ00/00159
- (22) International Filing Date: 17 August 2000 (17.08.2000)
- (25) Filing Language: English
- (26) Publication Language: English
- (30) Priority Data:  
337276 17 August 1999 (17.08.1999) NZ
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Constance Street, Hamilton (NZ).
- (81) Designated States (*national*): AE, AG, AL, AM, AT, AU,  
AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ,  
DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR,  
HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR,  
LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ,  
NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM,  
TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.
- (84) Designated States (*regional*): ARIPO patent (GH, GM,  
KE, LS, MW, MZ, SD, SI, SZ, TZ, UG, ZW), Eurasian  
patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European  
patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE,  
IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG,  
CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).
- Published:**  
— *With international search report.*
- For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*

WO 01/12844 A1

(54) Title: RAPID METHOD FOR MEASURING COMPLEX CARBOHYDRATES IN MAMMALIAN TISSUE

(57) Abstract: The invention discloses a rapid method of measuring complex carbohydrates in mammalian tissue, said method comprising the steps of: extracting a sample of tissue to be tested; forming a homogenous slurry of the sample with an aqueous solution; adding sufficient hydrolysing enzyme for ensuring complete hydrolysis of glycogen in the slurry; and measuring the concentration of glucose in the slurry. The method can be conducted post-mortem to assay the concentration at the time of slaughter. The invention further discloses the measurement of lactate concentrations. The method of the invention can be conducted in thirty minutes or less.

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/NZ00/00159

<b>A. CLASSIFICATION OF SUBJECT MATTER</b>												
Int. Cl. <sup>7</sup> C12Q 1/34, 1/54												
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Date of the actual completion of the international search 2 November 2000		Date of mailing of the international search report 14 NOV 2000										
Name and mailing address of the ISA/AU AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaustalia.gov.au Facsimile No. (02) 6285 3929		Authorized officer  PHILIPPA WYRDEMAN Telephone No: (02) 6283 2554										